BIOLOGICAL BULLETIN

ON THE INFLUENCE OF ELECTROLYTES UPON THE RATE OF RHYTHMIC MUSCULAR CON-TRACTIONS (PRELIMINARY COMMUNICATION).

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1. In a previous paper [1] I have suggested that the rate of beat of a rhythmically contracting tissue, such as the heart, may be connected with the velocities of the ions in the tissue-fluids, or in the medium in which the heart is suspended, by the following formula :

$$t = a \cdot \frac{u}{v(u + v)} + b,$$

where t is the interval between the beginning of one beat and the beginning of the next, u is the average velocity of the kations in the medium, v that of the anions in the medium and a and b are constants for any given heart.

2. The assumptions involved in the deduction of this formula are : (1) That the ions diffuse into the tissue in numbers proportional to their migration-velocities in water ; (2) that they give rise to a reversible chemical change in the tissue, a change consisting in the displacement of ions already present in the tissue in ion-proteid compounds by those which have newly entered, and that this reaction obeys Guldberg and Waage's mass-law and (3) that the ions thus displaced in each successive segment of tissue give rise to the contraction.

[1] *Pflüger's Arch. f. d. ges. Physiol.*, Bd. **110**, p. 610.

3. It occurred to me that, provided the above-mentioned assumptions were correct, the efficacy of a saline medium in maintaining the heart-beat at a normal rate should depend upon two factors, namely, the reversibility of the chemical changes inaugurated by it, and the value of $u/[v(u + v)]$ for the solution. Any solution bringing about only reversible changes (that is, for example, not containing heavy metals) and having the same value of $u/[v(u +v)]$, as Ringer's solution, should be equally efficacious in maintaining the heart-beat. Loeb has introduced the conception of Physiologically Balanced Salt Solutions.[1] It may be that as far as rhythmically contracting tissues are concerned the value of $u/[v(u + v)]$ may afford us a clue to the theoretical composition of such solutions.

4. I made up a Ringer solution with the following composition : $N/10$ NaCl $+ N/250$ KCl $+ N/200$ CaCl$_2$, which proved to be a good heart solution. The NaCl was made free from potassium by volatilizing the KCl out of Kahlbaum's C.P. NaCl, KCl having a lower boiling-point than NaCl. The average value of u for such a solution can be found by multiplying the velocity of each kation by its equivalent molecular concentration, and dividing the sum of these products by the sum of the concentrations. The value of $u/[v(u + v)]$ for this Ringer solution, calculated from the values for the ionic velocities glven in Landolt-Börnstein,[2] is 621.2×10^{-5}. A solution of LiNO$_3$, NH$_4$NO$_3$ and NaNO$_3$ having approximately the same value for $u/[v(u + v)]$ is one made up as follows : $.135 N$ LiNO$_3 + .03 N$ NH$_4$NO$_3 + .01 N$ NaNO$_3$ —the value of $u/[v(u + v)]$ (calculated from the table of ionic velocities quoted above) is 628×10^{-5}. This forms an excellent heart solution ; twenty-three variations of this solution were tested, keeping the concentration of the LiNO$_3$ constant, and varying that of the NH$_4$NO$_3$ and NaNO$_3$. In solutions 1–5 the LiNO$_3$ and NaNO$_3$ were kept at $.135 N$ and $.015 N$ respectively, and the NH$_4$NO$_3$ varied from $.03 N$ to $045 N$, increasing in concentration in each solution from 1 to 5 by $.005 N$. In solutions 6–9 the concentrations of the LiNO$_3$ and NH$_4$NO$_3$ were kept at $.135 N$

[1] *American Journal of Physiology*, Vol. III. (1900), p. 431, " Studies in General Biology," Decennial Publications of the University of Chicago, Vol. II., p. 590.
[2] *Physikalische-Chemische Tabellen*, 1905.

and .03N respectively, and that of the NaNO$_3$ was raised from 0.15N to .035N, differing by .005N in each successive solution.

Solution 10 was the calculated solution. In solutions 11–15 the concentrations of the LiNO$_3$ and NaNO$_3$ were kept at .135N and .015N respectively, while the concentration of the NH$_4$NO$_3$ was decreased from .02N in 11 to .000N in 15 by steps of .005N. In solutions 16–19 the concentrations of the LiNO$_3$ and NaNO$_3$ were kept at .135N and .015N respectively, while that of the NH$_4$NO$_3$ was raised from .031N in 16 to .034N in 19 by steps of .001N. In solutions 20–23 the concentrations of the LiNO$_3$ and NH$_4$NO$_3$ were kept at .135N and .03N respectively, and the concentration of the NaNO$_3$ raised from .016N to .019N in steps of .001N. Out of all the twenty-three solutions thus tried the only ones which approximated to the Ringer as sustainers of the heart-beat were the calculated solution (10) and solution 1 (.135N LiNO$_3$ + .03N NH$_4$NO$_3$ + .015N NaNO$_3$) in which $u/[v(u + v)] = 629.5$ — and these are the two solutions which approximate most closely to Ringer in the value of $u/[v(u + v)]$. Thus solution 11 (.135N LiNO$_3$ + .02N NH$_4$NO$_3$ + .015N NaNO$_3$) was much less efficacious than the calculated solution. On estimating the value of $u/[v(u + v)]$ for this solution the reason becomes clear, for its value is 615.1, a value departing comparatively widely from that for Ringer or for solution 10. The other solutions in which the NH$_4$NO$_3$ concentration was still further lowered (11–15) were still more deleterious. Raising the concentration of the NaNO$_3$ above that in solution 1 was also deleterious (solutions 6–9), and it will be found that in these solutions the value of $u/[v(u + v)]$ rises above 630, as might be expected; raising the concentration of the NH$_4$NO$_3$ (solutions 1–5) was still more deleterious.

In solution 10, to quote one experiment, a heart washed in NaCl and beating initially at a rate of 10 in 15 seconds, continued beating for 65 minutes; in 1, a heart beating initially at the same rate and treated in the same way as the above, and at the same time, beat for from 40–60 minutes; while a third heart, in Ringer, and beating initially at the same rate as the other two, beat for 90 minutes.

5. Thinking that perhaps the concentrations of these solutions were somewhat too high to be altogether favorable, I made up a series of six solutions with the concentration of $LiNO_3$ in each $.1N$ — one, solution 24, was calculated so as to give a value of $u/[v(u + v)]$ approximating to that in Ringer — its composition was $.1N LiNO_3 + .02N NH_4NO_3 + .015N NaNO_3$, and the value of $u/[v(u + v)]$ was 626.7. The remainder were solutions containing the same concentrations of $LiNO_3$ and $NaNO_3$, but varying in NH_4NO_3 concentration from $.02N$ (24) to $.025N$ (29). The most favorable solution was 24, the others departing more widely in the value of $u/[v(u + v)]$ from that for Ringer.

This new calculated solution formed a really excellent sustainer of the heart-beat, and by the addition of a trace (1 c.c. $N/40$ to 100 c.c. solution) of Na_2CO_3, to give a favorable alkalinity, a solution was obtained which was in every way as favorable a medium for the heart-beat as the Ringer to which the same amount of Na_2CO_3 had been added. Frogs' hearts beat in these solutions ($24 + Na_2CO_3$ and Ringer $+ Na_2CO_3$) for over two hours.

6. Thus the facts are in favor of the hypothesis, and we see that neither K, Ca or Cl ions are specific and essential for the heart-beat, nor, probably, Na ions — all that is necessary is to obtain a properly balanced solution containing no ions (such as heavy metals) which make irreversible compounds with proteids, and having the most favorable value of $u/[v(u + v)]$. Such a solution could be made up from any two or three salts whose values of $u/[v(u + v)]$ differed sufficiently widely in opposite directions from the value for Ringer. It is not inconceivable, of course, that a favorable heart solution may ultimately be found which contains a heavy metal — but one would not expect to find such a solution having the same value of $u/[v(u + v)]$ as Ringer, since the constants a and b (in the equation which I have quoted) would probably be altered by the heavy metal.

These experiments will be continued.

7. In the course of an investigation on the influence of temperature upon the rate of the heart-beat in *Ceriodaphnia* the results of which have appeared in the BIOLOGICAL BULLETIN,[1] I found that the rate of the heart-beat in *Ceriodaphnia* is practically constant

[1] Vol. X., p. 242 (1906).

at 21° C., provided the different individuals are taken from the same vessel, treated in the same way, and not previously exposed to any extreme temperatures.   With these precautions individuals differing widely in development will be found to have practically the same rate of heart-beat at 21°.   *Ceriodaphnia* can therefore be used as homogeneous material for the investigation of the influence of electrolytes upon the rate of the heat-beat — that is, the constants a and b in the equation

$$t = a \cdot \frac{u}{v(u + v)} + b$$

are practically the same for all the individuals, and the influence of each solution can be tested upon a fresh individual.   The number of beats was registered upon a revolving drum by tapping a key in a circuit containing a signal-magnet, and the time was taken with a stop-watch.

8. The following are the experimental results so far obtained, the "time of one beat" meaning the interval between the beginning of one beat and the beginning of the next.   The values of $u/[v(u + v)]$ are calculated from the table of ionic-velocities to which I have previously referred.   In every case the organisms were first washed in tap-water, then placed in about 50 cc. of the solution for 10 minutes and then placed, with a drop of solution, on a glass plate kept accurately at 21° by means of a stream of water running underneath — in 10 minutes the rate of beat was noted.

Solution.	$u/[v(u+v)]$	Time of one beat.	Value of a when $b=1.7232$.
$N/10$ LiNO$_3$	567.7×10^{-5}	.3 sec.	356.4
$N/10$ NaCl	611.6×10^{-5}	.150 "	306.6
Ringer	621.2×10^{-5}	.183 "	30 .9
100 c.c. Ringer + 2 cc. $N/10$ KCl	624.1×10^{-5}	.185 "	305.75
$N/10$ MgCl$_2$	631.4×10^{-5}	.2 "	304.6
40 c.c. $N/10$ NaCl + 10 c.c. $N/10$ KCl	645.9×10^{-5}	.238 "	303.7
30 c.c. $N/10$ NaCl + 20 c.c. $N/10$ KCl	677.8×10^{-5}	.353 "	306.6
30 c.c. $N/10$ LiNO$_3$+ 20c.c. $N/10$ NH$_4$CNS	725.1×10^{-5}	.375 "	289.4
$N/10$ NH$_4$CNS	937.6×10^{-5}	.5 "	237.1

Thus we see that the value of a is constant for values of $u/[v(u + v)]$ between and including 611.6×10^{-5} and 677.8×10^{-5}. Above and below those values it is variable.

9. It is evident that upper and lower limiting values of $u/[v(u + v)]$ exist between which sustained beats are possible.

The existence of the lower limit is evident from a consideration of the equation

$$t = \frac{au}{v(u + v)} + b,$$

for since b has a negative sign, when

$$\frac{u}{v(u + v)} = -\frac{b}{a}$$

then the time of one beat is zero, and for still lower values of $u/[v(u + v)]$ the time of one beat would be negative, which is, of course, impossible. The equation

$$\frac{u}{v(u + v)} = -\frac{b}{a},$$

therefore gives us a theoretical lower limit, in this case 562×10^{-5}. Before this, however, as the experiment with $LiNO_3$ shows, the beats begin to fuse — for the beats take a certain time to traverse the heart, and this interval may be greater than that between the beginnings of two beats. The upper limit is possibly the expression of the fact that ions must reach the minimum number necessary to displace those in combination with the proteids (the "threshold-number") before they have time to diffuse away through the tissue. Before this point, however, irregularities appear, as in the last two experiments in the table above. The beats soon stop in these extreme solutions. Thus in KCl or in 20 c.c. $N/10$ NaCl $+$ 30 c.c. $N/10$ KCl the beats are only sustained for about 5 minutes at $21°$ — in the latter solution the value of $u/[v(u + v)]$ is 706.9, and we notice that it is just about at this value of $u/[v(u + v)]$ that irregularities begin to appear in the value of the constant a. Hence the limits for *Ceriodaphnia* are probably 570×10^{-5} and 700×10^{-5}. Any solution in which $u/[v(u + v)]$ lies between these values, and which does not contain any substance forming irreversible proteid compounds, should sustain the heart-beat in this organism — solutions having values of $u/[v(u + v)]$ falling outside these limits could not sustain the heart-beat.

10. By mixing a solution having a value of $u/[v(u + v)]$ below the lower limit for the rhythmically contracting tissue in question

with one having a value of $u/[v(u + v)]$ above the upper limit, a solution could evidently be obtained which would sustain the heartbeat. In this way many salt-antagonisms could be obtained. Thus I have found that the heart of *Ceriodaphnia* will not beat in $N/10$ KCl or in 20 c.c. $N/10$ NaCl + 30 c.c. $N/10$ KCl, but will beat in 30 c.c. $N/10$ NaCl + 20 c.c. $N/10$ KCl, because the two former solutions have values of $u/[v(u + v)]$ lying above the upper limit for this rhythmically beating tissue, while the last-mentioned solution has a value of $u/[v(u + v)]$ lying between the upper and lower limits. If we were considering the question as one of toxicity, we should say that NaCl *antagonized* KCl. The possible reason for the antagonism is now clear.

11. The values of the upper and lower limits for rhythmical contractions in a tissue will obviously depend upon the constants a and b for that tissue in the equation

$$t = a \cdot \frac{u}{v(u + v)} + b.$$

These constants will be different in different tissues, and need not be the same for different parts of the same tissue. Thus, in the case of *Gonionemus*, it is only necessary to suppose that the limits for the center lie very close on either side of the value of $u/[v(u + v)]$ for NaCl, while the limits for the margin are somewhat farther apart, to obtain a possible explanation of the fact that the center will beat in NaCl solution but not in NaCl solution to which KCl or $CaCl_2$ have been added, while the *margin* will continue to beat in such solutions.[1] In this way many of the apparently specific effects of certain ions upon rhythmically contracting tissues may possibly be reduced to a general explanation.

12. Experiments will be continued with a view to obtaining further evidence as to the existence of upper and lower limits in the value of $u/[v(u + v)]$, above and below which rhythmic contractions are impossible, and also further experiments upon the degree to which the crustacean heart obeys the formula

$$t = a \cdot \frac{u}{v(u + v)} + b.$$

[1] Loeb, *American Journal of Physiology*, Vol. III. (1900), p. 383.